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**THE DISTRIBUTION OF BROMIDE
AND CHLORIDE IN TISSUES
AND BODY FLUIDS**

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

1936

By
EVERETT GEORGE WEIR

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CHICAGO, ILLINOIS**

Reprinted from
THE JOURNAL OF BIOLOGICAL CHEMISTRY
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THE DISTRIBUTION OF BROMIDE AND CHLORIDE IN TISSUES AND BODY FLUIDS

By EVERETT G. WEIR

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Research of the University of Chicago, Chicago)*

(Received for publication, May 24, 1939)

A review of the literature dealing with the distribution of bromides between the blood and various body fluids and tissues, after bromide administration, reveals a wide variety of experimental results. (a) The distribution ratio of Br^- between red blood cells and serum has been reported as lower (1), the same as (2), and higher (3) than the corresponding Cl^- ratio. (b) It has been claimed (4) and denied (5) that the kidneys preferentially excrete Cl^- over Br^- . (c) Many workers have reported that certain tissues can concentrate bromide to an extent greater than the blood bromide concentration. There is no agreement, however, as to which tissues do this. The intestine (6), striated muscle (7), and brain (8) have been implicated. Still other workers have found no evidence of any "tissue affinity" for bromide. (d) Only in the demonstration that a barrier exists to the free passage of Br^- into the spinal fluid does there seem to be agreement among reported results.

The present investigation was undertaken with the purpose of (a) reconciling, if possible, the discrepancies of past work, and (b) presenting quantitative data on the proportion of halide-containing fluid of various tissues. An excellent study of bromide distribution in tissues and body fluids carried out independently has been reported by Wallace and Brodie (9). Our results are in agreement with theirs. In addition, we shall present the results of the injection of large amounts of bromide-containing isotonic salt solutions on the extracellular fluid of muscle and skin.

EXPERIMENTAL

Two types of experiments were carried out: (a) Those in which NaBr was administered by mouth, following which various body fluids and tissues were analyzed for their chloride, bromide, and water contents. The serum pH and tissue fats were also determined. (b) Those in which isotonic solutions containing sodium bromide were injected intravenously. Analyses of blood, striated muscle, and skin were made before and after the injection of the sodium bromide solution.

The experiments were carried out on dogs which were under barbital anesthesia during all operative procedures. Samples of blood and tissue were obtained and prepared for analysis according to the methods described by Hastings and Eichelberger (10).

The water content of the fluids and tissues was determined by drying weighed quantities at 105°. Tissue determinations were made in quadruplicate, fluid determinations in duplicate.

Serum pH was determined by the colorimetric method of Hastings and Sendroy (11). Total tissue fat was determined according to the procedure described by Hastings and Eichelberger (10).

Determination of Bromide—Examination of the reasons for the discrepancies in results encountered in the literature led us to believe that the methods employed were sometimes at fault. The electrotitrimetric method, applied by Hastings and van Dyke to blood serum and cells, was found to be inaccurate when the bromide content was low. This was due to the fact that they used tungstic acid filtrates of cells which contained organic material capable of reacting with silver. A method was, therefore, developed for obtaining tissue chlorides and bromides in a solution free from organic material. In principle, the method consists of (a) formation of the silver salts of chloride and bromide and oxidation of the organic material; (b) decomposition of the silver salts and their distillation; (c) electrometric titration of Br^- ; (d) determination of total halide and hence the chloride by difference.

The halides of the dried fat-extracted tissue (approximately 0.4 gm.), obtained after the determination of water and fat, were converted into their silver salts by digestion with excess silver nitrate and nitric acid according to the recommendations of Sunderman and Williams for analysis of dried tissues (12). The

mixture of AgCl and AgBr formed was allowed to settle to the bottom of the Pyrex tube in which digestion was carried out and the supernatant liquid was pipetted off, with a fine tipped pipette. The precipitate was washed with distilled water until the wash solution was silver-free. Three washings were found to be adequate.

The apparatus used in the decomposition of the silver halides, shown in Fig. 1, is modified after Bell and Doisy (13), and so

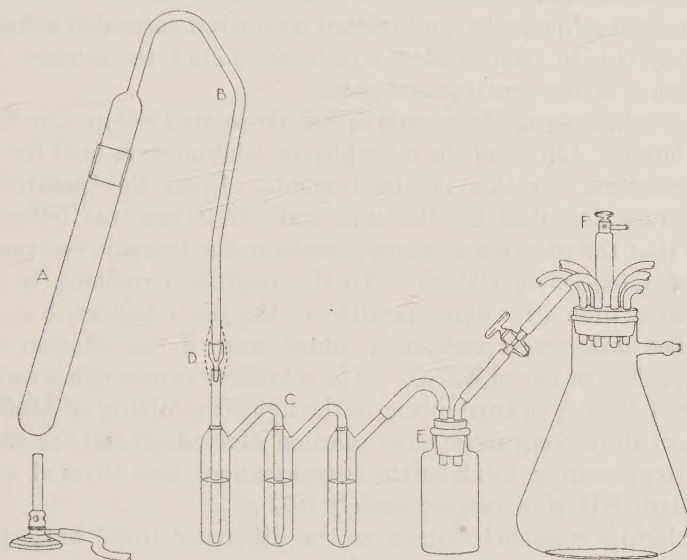


FIG. 1. Distilling apparatus employed in the decomposition of silver halides. A, Pyrex digestion tube; B, connecting tube; C, absorbing vessel; D, glass reducer held fixed by rubber tubing (dotted lines); E, safety bottle; F, valve for controlling suction centrally.

constructed that the halogens and halogen acids formed when the AgCl and AgBr are decomposed are drawn by suction through the absorbing vessels without having come into contact with organic material.

The washed silver halides were decomposed in the original digestion tube by adding 0.1 gm. of MnO_2 and 3 cc. of concentrated H_2SO_4 . The digestion tube was then attached to the distilling apparatus, and the mixture was maintained at its boiling point

for 30 to 40 minutes. The halogens liberated are absorbed in a receiving vessel containing 15 cc. of 0.1 N Na_2SO_3 in 4 N NaOH. In the course of the reaction, some Cl_2 and Br_2 are formed together with HCl and HBr. The free halogens are reduced principally by the SO_2 liberated from the H_2SO_4 and to a lesser extent by the Na_2SO_3 in the receiving mixture.

When the distillation was complete, the contents of the receiving vessels were quantitatively washed into 125 cc. Erlenmeyer flasks, neutralized with clear, concentrated HNO_3 , methyl orange being used as indicator, and treated with a hot saturated solution of $\text{Ba}(\text{NO}_3)_2$ to remove $\text{SO}_4^{=}$ which was found to decrease the sharpness of the bromide end-point.

The solution was then made up to 100 cc. in a volumetric flask and filtered. 50 cc. of the clear filtrate solution were used for the electrometric titration of the bromide. From this point, the procedure described by Hastings and van Dyke was followed, save that the titration was only carried to the bromide end-point.

Determination of Chloride—To the mixture remaining in the digestion tube after decomposition of the silver halides, 3 cc. of distilled water were cautiously added, and, if the solution was pink, a drop or two of $\text{K}_2\text{C}_2\text{O}_4$. The solution was now either water-clear or slightly opaque owing to the limited solubility of MnSO_4 . 3 cc. of 10 per cent ferric alum solution were added and the silver ion (representing total halide concentration) was titrated with standard NH_4SCN (approximately 0.02 N).

Chlorides were determined as the difference [total halide] — [bromide].

Determinations on body fluids for Cl and Br were made in triplicate; on tissues, determinations were made in quadruplicate.

When known amounts of bromide were added to tissues and body fluids, it was found that a slight end-point correction was necessary to yield correct results. The magnitude of these bromide corrections is given in Table I. Total halide recovery in tissues and body fluids was satisfactory without correction.

Results

Distribution of Bromide and Chloride in Blood—Although bromide added to blood *in vitro* is distributed between serum and cells in a quantitatively predictable manner (Hastings and van Dyke

(14)), it has been reported that bromide, given by mouth, is sometimes concentrated to a surprising degree in the red blood cells (van Dyke and Hastings (3)). These observations have been confirmed by Mishkis (15) using the same analytical procedure. Mason, using ashed samples instead of tungstic acid filtrates, found bromide ratios which are only slightly higher than the corresponding chloride ratios (16).

Experiments were, therefore, carried out to reexamine this question (Table II). It was found that the bromide distribution ratio between serum and cells was 6 per cent higher than the cor-

TABLE I
End-Point Corrections in Bromide Titrations

Experiment No.	0.01 N AgNO ₃		Difference
	Calculated	Found	
Body fluids with added NaBr			
	cc.	cc.	cc.
1	0.501	0.484	+0.017
2	1.003	1.008	-0.005
3	1.505	1.545	-0.040
4	2.006	2.040	-0.034
5	2.507	2.575	-0.068
Tissues with added NaBr			
1	0.50	0.37	+0.13
2	1.00	0.92	+0.08
3	1.50	1.44	+0.06
4	2.00	1.87	+0.13

responding chloride ratio. This agrees with the observations made by Mason. The results previously reported by van Dyke and Hastings are probably incorrect and were due to the use of a tungstic acid filtrate instead of ashed material.

Distribution of Bromide between Serum and Peritoneal Fluid—Sufficient peritoneal fluid was obtained from two dogs after sodium bromide administration by mouth to permit the comparison of the bromide concentration therein with that of the serum. The ratios of the bromide concentrations were also compared with the chloride ratios (Table III). Within the limits of experimental

error, the chloride and bromide ratios agreed in magnitude. It is of interest that the ratios averaged 1.03 instead of 0.95, which one would expect to find at equilibrium between serum and a protein-free ultrafiltrate. The peritoneal fluid in our experiments contained approximately 3 per cent of protein.

TABLE II
*Distribution of Bromide and Chloride in Blood at Varying Times
Following Sodium Bromide Administration*

Dog No.	pH _s	[Cl ⁻] _s	[Cl ⁻] _c	[Br ⁻] _s	[Br ⁻] _c	[Cl ⁻] _c [Cl ⁻] _s	[Br ⁻] _c [Br ⁻] _s	Time since last dose of NaBr	Method of NaBr administration
		mM per kg. H ₂ O	mM per kg. H ₂ O	mM per kg. H ₂ O	mM per kg. H ₂ O				
6	7.37	74.0	58.2	55.2	45.6	0.79	0.83	20 hrs.	Orally
	7.36	70.3	55.7	51.8	40.5	0.80	0.78	3 days	"
	7.38	85.8	56.5	38.9	27.5	0.66	0.710	9 "	"
	7.52	93.5	63.3	30.3	22.6	0.68	0.750	15 "	"
7	7.42	100.1	69.4	23.1	16.9	0.69	0.73	20 hrs.	"
	7.42	91.8	71.3	34.2	27.1	0.79	0.78	22 days	"
9	7.39	91.7	64.2	24.6	17.6	0.70	0.72	4 "	"
10	7.36	91.0	66.1	34.2	26.6	0.73	0.78	6 "	"
8	7.45	94.8	62.5	30.3	22.6	0.66	0.750	0.5 hr.	Intravenously
13	7.34	76.5	55.1	45.4	36.2	0.72	0.80	0.6 "	"

TABLE III
Distribution of Chloride and Bromide between Serum and Peritoneal Fluid

Dog No.	[Cl ⁻] _s	[Cl ⁻] _{p.f.}	[Br ⁻] _s	[Br ⁻] _{p.f.}	[Cl ⁻] _s [Cl ⁻] _{p.f.}	[Br ⁻] _s [Br ⁻] _{p.f.}
	mM per kg. H ₂ O	mM per kg. H ₂ O	mM per kg. H ₂ O	mM per kg. H ₂ O		
6	93.5	90.7	30.3	29.5	1.03	1.03
12	85.9	83.5	34.7	34.9	1.04	0.99

Distribution of Bromide between Serum and Cerebrospinal Fluid—
In view of the accurate observations previously made on the distribution of bromide between serum and cerebrospinal fluid (15-17), only two such experiments are reported here (Table IV). In agreement with the above authors, it was found that the concentration of bromide in the cerebrospinal fluid is appreciably less than one would expect on the basis of the total halide distribution.

Distribution of Bromide between Serum and Tissues. Bromide Given by Mouth—The results of comparing the bromide distribution in different tissues following sodium bromide administration by mouth are given in Table V. The analytical results have been expressed in terms of units per kilo of fat-free tissue. From these have been calculated the percentage replacement of chloride by bromide and the extracellular fluid of the tissues in gm. per kilo of tissue.

The conclusion reached from an examination of these data is that none of the tissues examined can be regarded as having a special preferential affinity for bromide rather than chloride. Indeed, except for the brain and the spinal fluid, there is good agreement among the different tissues and blood plasma in the degree to which the chloride has been replaced by bromide.

TABLE IV

Distribution of Chloride and Bromide between Serum and Spinal Fluid

Dog No.	[Cl ⁻] _s	[Cl ⁻] _{s.f.}	[Br ⁻] _s	[Br ⁻] _{s.f.}	$\frac{[\text{Cl}^-]_s}{[\text{Cl}^-]_{s.f.}}$	$\frac{[\text{Br}^-]_s}{[\text{Br}^-]_{s.f.}}$
	<i>mM per kg. H₂O</i>	<i>mM per kg. H₂O</i>	<i>mM per kg. H₂O</i>	<i>mM per kg. H₂O</i>		
11	77.4	95.5	43.7	36.7	0.81	1.19
12	85.9	103.7	34.7	28.0	0.83	1.24

Assuming that the halides are similarly distributed throughout the body, and that the halide ions are confined to the extracellular phase of tissues, the relative proportion of extracellular fluid in the different tissues has been estimated from both the chloride and bromide data. The method of calculation has been thoroughly described by Hastings and Eichelberger, and will not be repeated here.

It was found that there was agreement between the amounts of extracellular fluid per kilo of tissue calculated, on the one hand, from chloride data and, on the other, from bromide data. From these observations, it may be concluded that bromide may be substituted for chloride to estimate the proportion of extracellular fluid in tissues. A method for the determination of total extracellular fluids, based on the administration of bromide, has recently been successfully developed by Brodie, Leshin, and Brand (18).

TABLE V

Distribution of Chloride and Bromide between Blood and Tissues Following Oral Administration of Sodium Bromide

Dog No.	Sample	H ₂ O	[Cl ⁻]	[Br ⁻]	Extracellular fluid calculated from			Per cent replacement, [Br ⁻]: [Cl ⁻ + Br ⁻]
					[Cl ⁻]	[Br ⁻]	[Cl ⁻ + Br ⁻]	
		gm. per kg.	mM per kg.	mM per kg.	gm. per kg.	gm. per kg.	gm. per kg.	
6	Serum (pH 7.52)	923.0	86.3	27.8				24.5
	Red blood cells	674.0	42.7	15.2				26.3
	Peritoneal fluid	961.4	87.2	28.3				24.5
	Striated muscle	768.5	14.5	4.5	147	141	146	23.7
	Whole stomach	802.2	52.1	20.9	530	656	561	28.6
	Duodenum	792.5	35.8	12.1	364	379	369	25.2
9	Serum (pH 7.39)	911.8	83.6	22.4				21.1
	Red blood cells	646.2	41.5	11.3				21.5
	Striated muscle	770.6	15.2	4.2				21.7
	Smooth (stomach) muscle	798.7	40.6	12.5	421	483	435	23.5
	“ (uterus) “	788.2	42.2	11.9	437	460	444	22.0
	Mucosa (stomach)	777.6	50.4	14.4	522	556	531	22.2
10	Serum (pH 7.36)	919.9	83.7	31.4				27.6
	Red blood cells	663.9	43.9	17.7				28.7
	Striated muscle	753.3	11.4	5.9	118	164	132	34.0
	Smooth (stomach) muscle	782.2	43.7	19.4	456	539	480	30.7
	Mucosa “	734.3	50.1	23.3	523	647	559	31.7
	“ “	734.3	50.1	23.3	523	647	559	31.7
11	Serum (pH 7.36)	920.5	71.2	40.2				36.0
	Spinal fluid	987.0	94.3	36.2				27.7
	Liver	761.4	31.1	17.3	375	376	375	35.7
	Duodenum	790.1	29.6	16.0	363	348	358	35.1
	Skin	726.1	50.3	31.0	618	674	640	38.2
	Kidney	791.4	35.8	19.1	440	415	432	34.8
	Bone	256.0	22.2	13.3	273	289	280	37.7
	Cerebrum	856.2	27.6	9.6	339	209	243	25.8
12	Serum (pH 7.17)	905.2	77.8	31.4				28.5
	Peritoneal fluid	958.0	80.8	33.4				29.5
	Spinal fluid	987.4	102.4	26.8				21.5
	Striated muscle	768.7	14.7	6.6	163	181	168	31.9
	Kidney	784.3	45.7	17.8	511	487	502	28.0
	Duodenum	793.1	32.0	13.7	357	375	361	30.0
	Skin	713.9	53.4	20.9	596	572	588	28.1
	Liver	696.3	23.7	10.0	265	274	266	29.7

TABLE VI

Distribution of Chloride and Bromide between Blood and Tissues Following Intravenous Injection of Isotonic Solutions Containing NaBr

Dog 13, weight 16 kilos, injected with 2000 cc. of 129 mm NaBr + 25 mm NaHCO₃; Dog 17, weight 9.6 kilos, injected with 1500 cc. of 144 mm NaBr + 10 mm HCl; Dog 18, weight 9.6 kilos, injected with 1600 cc. of 142 mm NaBr + 12 mm HCl; Dog 15, weight 18 kilos, injected with 2300 cc. of 114 mm NaBr + 40 mm NaHCO₃; Dog 16, weight 21 kilos, injected with 3400 cc. of 114 mm NaBr + 40 mm NaHCO₃.

Dog No.	Sample	H ₂ O	[Cl ⁻]	[Br ⁻]	Extracellular fluid calculated from			Per cent replacement, [Br ⁻]:[Cl ⁻ + Br ⁻]
					[Cl ⁻]	[Br ⁻]	[Cl ⁻ + Br ⁻]	
		gm. per kg.	mM per kg.	mM per kg.	gm. per kg.	gm. per kg.	gm. per kg.	
13	Control							
	Serum (pH 7.40)	915.4	108.7					
	Striated muscle	771.3	19.73		158			
	Final							
	Serum (pH 7.34)	938.4	71.8	42.6				37.2
17	Striated muscle	780.1	13.2	7.9	164	166	165	37.4
	Control							
	Serum (pH 7.38)	925.8	110.6					
	Striated muscle	764.4	23.2		181			
	Skin	682.7	83.8		654			
18	Final							
	Serum (pH 7.31)	944.0	79.0	33.7				30.1
	Striated muscle	786.6	19.0	6.3	214	166	203	25.1
	Skin	727.5	63.9	25.5	718	670	712	28.5
	Control							
15	Serum (pH 7.32)	918.0	108.7					
	Striated muscle	763.9	23.3		159			
	Skin	709.4	88.3		708			
	Final							
	Serum (pH 7.19)	944.0	71.7	49.1				40.6
16	Striated muscle	771.1	14.9	9.4	186	172	181	38.7
	Skin	741.4	59.4	39.7	742	726	738	40.0
	Control							
	Serum (pH 7.37)	919.0	111.0					
	Striated muscle	770.5	29.5		232			
15	Skin	683.4	90.8		714			
	Final							
	Serum (pH 7.45)	937.7	75.1	32.6				30.2
	Striated muscle	790.5	22.7	7.7	269	210	252	25.5
	Skin	745.2	56.7	23.3	673	636	662	29.1

TABLE VI—*Concluded*

Dog No.	Sample	H ₂ O	[Cl ⁻]	[Br ⁻]	Extracellular fluid calculated from			Percent replacement, [Br ⁻]/([Cl ⁻ + Br ⁻])
					[Cl ⁻]	[Br ⁻]	[Cl ⁻ + Br ⁻]	
		gm. per kg.	mM per kg.	mM per kg.	gm. per kg.	gm. per kg.	gm. per kg.	
16	Control							
	Serum (pH 7.31)	924.0	110.5					
	Striated muscle	788.2	25.8		203			
	Skin	732.8	33.1		654			
	Final							
	Serum (pH 7.40)	937.5	69.1	40.3				36.8
	Striated muscle	790.5	16.8	8.1	217	177	206	32.5
	Skin	764.5	52.8	28.7	680	626	664	35.2

Whether or not some tissue cells, as, for example, those of the stomach mucosa, are permeable to bromide as well as chloride ions is, of course, not answered by these observations.

Bromide Injected Intravenously A series of experiments was carried out in which large quantities of isotonic solutions containing sodium bromide were injected intravenously. After a period of 45 to 60 minutes following the completion of injection to permit the establishment of equilibrium, blood and tissues were removed for analysis. (Control samples of tissue and blood were taken prior to the injection of the bromide solution.) The solutions injected were of three kinds: (a) those which contained 25 mM of NaHCO₃ in order to minimize alterations in the acid-base balance; (b) those which contained HCl in order to favor the production of an acidosis; and (c) those which contained 40 mM of NaHCO₃ in order to favor the production of an alkalosis. It should be said that the acid-base changes, as measured by the pH of the blood, were not large in any experiment. Illustrative experiments are listed in Table VI.

The principal point of interest in these experiments centers around the calculated values for extracellular fluid. The injection of the salt solutions resulted in an increase in the proportion of extracellular fluid in the striated muscle and skin as calculated

from the chloride and total halide data. Corresponding calculations, based on the bromide data, lead to slightly lower values for extracellular fluid, which are probably to be attributed to failure to attain equilibrium between the bromide of the blood and tissues.

DISCUSSION

Except in the case of the cerebrospinal fluid and the brain, the replacement of chloride by bromide seems to be essentially the same in all the tissues studied. It would seem reasonable to conclude, therefore, that (a) there is no special affinity for bromide in tissues, and (b) bromide diffuses freely into and from tissues wherever chloride is present until equilibrium is established.

The results observed in the brain and cerebrospinal fluid require special mention. It will be noted that the per cent replacement in both was lower than in blood or other tissues. Furthermore, the replacement values in cerebrospinal fluid and brain agree essentially with each other. It would seem not unreasonable to suppose, therefore, that cerebrospinal fluid may be regarded as in equilibrium with the extracellular fluid of brain tissue, at least, in so far as halide ions are concerned. In this sense, therefore, cerebrospinal fluid may be considered representative of the extracellular fluid of brain. In this respect, our results and the conclusions drawn from them are in agreement with those reported by Wallace and Brodie (17).

For suggesting this problem and directing its execution the author is indebted to Dr. A. Baird Hastings. An expression of appreciation is extended to Dr. Lillian Eichelberger for her generous interest and aid during this work.

SUMMARY

1. The distribution of bromide and chloride in tissues and body fluids has been determined after the oral and intravenous administration of sodium bromide.

2. In agreement with the work of Wallace and Brodie, bromide has been found to replace chloride uniformly throughout the tissues and fluids of the body except for the brain and cerebrospinal fluid.

3. Estimates of the extracellular fluid of tissues have been made from their bromide and chloride contents.

BIBLIOGRAPHY

1. Guillaumin, C., *Compt. rend. Soc. biol.*, **113**, 1429 (1933).
2. Palmer, J. W., and Clarke, H. T., *J. Biol. Chem.*, **99**, 435 (1932-33).
3. van Dyke, H. B., and Hastings, A. B., *J. Biol. Chem.*, **92**, 27 (1931).
4. Hastings, A. B., Harkins, H. N., and Liu, S. K., *J. Biol. Chem.*, **94**, 681 (1931-32).
5. von Frey, E., *Z. exp. Path. u. Therap.*, **8**, 29 (1910).
6. Appelmans, M., *Arch. internat. pharmacod.*, **31**, 231 (1925).
7. Toxopeus, M., *Arch. exp. Path. u. Pharmacol.*, **149**, 263 (1930).
8. Purjesz, B., Berkesy, L., and Gonezi, K., *Arch. exp. Path. u. Pharmacol.*, **173**, 553 (1933).
9. Wallace, G. B., and Brodie, B. B., *J. Pharmacol. and Exp. Therap.*, **65**, 214 (1939).
10. Hastings, A. B., and Eichelberger, L., *J. Biol. Chem.*, **117**, 73 (1937).
11. Hastings, A. B., and Sendroy, J., Jr., *J. Biol. Chem.*, **61**, 695 (1924).
12. Sunderman, F. W., and Williams, P., *J. Biol. Chem.*, **92**, 99 (1931).
13. Bell, R. D., and Doisy, E. A., *J. Biol. Chem.*, **45**, 427 (1920-21).
14. Hastings, A. B., and van Dyke, H. B., *J. Biol. Chem.*, **92**, 13 (1931).
15. Mishkis, B. S., Thesis, University of Chicago (1932).
16. Mason, M. F., *J. Biol. Chem.*, **113**, 61 (1936).
17. Wallace, G. B., and Brodie, B. B., *J. Pharmacol. and Exp. Therap.*, **65**, 220 (1939).
18. Brodie, B. B., Leshin, S., and Brand, E., *Proc. Am. Soc. Biol. Chem.*, *J. Biol. Chem.*, **128**, p. xi (1939).

